

WO 98/55623

PCT/NL98/00336

25

CLAIMS

1. A ribosome-inactivating protein comprising a single chain protein, the protein being characterised by having a molecular weight of about 26,000 daltons by polyacrylamide gel electrophoresis under reducing and non-reducing conditions, a pI of about 9.0, and further comprising an amino-terminal amino acid residue sequence having at least 50% homology with the following amino acid sequence:

Y N T V S F N L G E A Y E Y P T F I Q D L R N E L A K G T P (SEQ ID No. 1),

or a biologically active fragment or equivalent of said protein having ribosome inactivating properties and/or having an amino acid sequence homology of at least 50% with said protein and/or comprising at least 5 contiguous amino acids of the amino acid sequence of said protein.

2. The protein of claim 1, said protein further being characterised by having an IC_{50} of about 0.04×10^{-11} M in a rabbit reticulocyte lysate system.

3. The protein of claim 1 or 2, said sequence homology being at least 65%, especially at least 75%, and/or said part comprising at least 8, especially at least 10 contiguous amino acids.

4. The protein of any one of claims 1-3, wherein said fragment comprises an amino acid sequence having at least 50%, especially at least 65% homology with the amino acid sequence of SEQ ID No. 9, and/or said fragment comprises at least 8, especially at least 10 contiguous amino acids of the amino acid sequence of SEQ ID No. 9.

5. The protein of any one of claims 1-4, wherein said protein can be isolated from a *Bougainvillea* species, especially *B. spectabilis* Willd.

6. A conjugate comprising the ribosome-inactivating protein of any one of claims 1-5 or fragment thereof, linked to a ligand to form a toxin-ligand conjugate.

7. The conjugate of claim 6, wherein said ligand comprises an immunoglobulin, hormone, growth factor, peptide or non-peptide ligand.

8. The conjugate of claim 7, wherein said immunoglobulin is a monoclonal antibody or single-chain monoclonal antibody, or a fragment thereof such as Fab, F(ab')₂, Fv, or other fragment which retains the antigen binding function of the parent antibody.

BEST AVAILABLE COPY

WO 98/55623

PCT/NL98/00336

26

9. A pharmaceutical composition comprising the ribosome-inactivating protein of any one of claims 1-5 or the conjugate of any one of claims 6-8, together with a pharmaceutically acceptable carrier or adjuvant.
10. The pharmaceutical composition of claim 9, wherein the pharmaceutically acceptable carrier or adjuvant is human serum albumin, albumin, an ion exchanger, alumina, lecithin, a buffer substance, salt or electrolyte.
11. An isolated oligonucleotide or polynucleotide sequence encoding a protein according to any one of claims 1-5, or a conjugate according to any one of claims 6-8, or part of thereof comprising a sequence of at least 15, especially at least 24 nucleotides.
12. A recombinant vector comprising an oligonucleotide or polynucleotide sequence according to claim 10 or 11.
13. The recombinant vector of claim 12, further comprising transcriptional and translational control sequences operably linked to the oligonucleotide sequence encoding the ribosome-inactivating protein.
14. A host cell transfected with a recombinant vector of claim 12 or 13.
15. A method for the recombinant expression of bouganin comprising transfecting a host cell with an expression vector comprising an oligonucleotide sequence encoding the bouganin amino acid sequence or an active fragment thereof, growing the transfected host cells, inducing the transfected host cells to express recombinant bouganin and isolating the expressed recombinant bouganin.
16. The method of claim 15, wherein said host cell is a bacterium, a plant cell, or a yeast.
17. A method for producing a recombinant bouganin-ligand fusion protein comprising transfecting a host cell with an expression vector comprising a nucleotide sequence encoding the bouganin amino acid sequence operably linked with a nucleotide sequence which encodes a ligand, growing the transfected host cells, inducing the transfected host cells to express the recombinant bouganin-ligand fusion protein, and isolating the expressed recombinant fusion protein.
18. The method of claim 17, wherein said host cell is a bacterium, a plant cell, or a yeast.

BEST AVAILABLE COPY

WO 98/55623

27

PCT/NL98/00336

19. The method of claim 17 or 18, wherein the ligand is a large molecular weight protein, a small molecular weight protein, a polypeptide, or a peptide-ligand.
20. The method of claim 19, wherein the ligand is an immunoreactive ligand.
21. The method of claim 20, wherein the immunoreactive ligand is an antigen-recognizing immunoglobulin, or an antigen-recognizing fragment thereof, a chimeric antibody, a bifunctional antibody, a hybrid antibody or a single chain antibody.
22. The method of claim 21, wherein the antigen recognizing fragment is a Fab', F(ab')₂, Fv or Fab fragment of an immunoglobulin.
23. A method for killing a target cell comprising contacting the target cell with an effective amount of a toxin-ligand conjugate of any one of claims 6-8, wherein the ligand specifically binds to or reactively associates with a receptor moiety on the surface of the target cell, for a time sufficient to kill the target cell.
24. The method of claim 23, wherein the ligand comprises an immunoglobulin, adhesion molecule, or a polypeptide, peptide or non-peptidyl ligand.
25. The method of claim 24, wherein the immunoglobulin is an antigen binding fragment, a chimeric antibody, a humanized antibody, a bifunctional antibody, a hybrid antibody, or a biological fragment retaining the ligand binding capacity.
26. A pharmaceutical composition comprising a polynucleotide according to claim 11, or encoding the ribosome-inactivating protein of any one of claims 1-5, together with a pharmaceutically acceptable carrier, optionally together with a targeting device.

BEST AVAILABLE COPY